

Maize (*Zea Mays* L) Landraces from the Southern Region of Brazil: Contamination by *Fusarium* sp, Zearalenone, Physical and Mechanical Characteristics of the Kernels

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ABSTRACT

*This work had as objectives the study of the physical and mechanical characteristics of maize kernels in relation to the contamination by *Fusarium* sp and by zearalenone in twenty landraces of maize from the southern region of Brazil. From the analyzed samples, 60 % has been considered to have a hard endosperm type and 40 % an intermediary one. A correlation between the physical and mechanical variables was observed as an indication that the higher is the proportion of hard endosperm, more dense will be the kernel and more force for its rupture will be necessary. The level of contamination by *Fusarium* sp was between 5.5 and 24.75% among the analyzed grains, correlating positively with the flotation index, indicating that the landraces of maize with a softer endosperm can present a higher contamination by this genus. The presence of zearalenone was verified in 75 % of the samples, in concentrations varying from 50 to 640 µg kg⁻¹.*

Key words: fungi, texture of endosperm, corn landraces, mycotoxins

INTRODUCTION

The maize is one of the most cultivated and consumed cereals in the world (Garcia et al., 2006) with an important role as human staple food, mainly in less developed countries (Anderson et al., 2000).

Maize landraces or local varieties are cultivated and used by traditional communities with peculiar characteristics that are the uniformity of the grains and plants and its genetic purity (Zeven, 2002).

The hardness and the rupture force of the cereals are related as properties that must be taken into account by the farmer at the moment of the choice of the maize variety to be planted (Pomeranz et al., 1984). The importance of these properties of the grain is due to the fact that the lower is the disruption of the kernel pericarp, lower will be the contamination levels by microorganisms, especially fungi (Mantovani and Sources, 1989). The presence of insects can increase the infection of the cereals by *Fusarium* sp through the

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following mechanisms:(a) compromising the external protection of the grains and the plant tissues, (b) allowing that hyphae of the fungus penetrates and has access to the grains' nutrients and, (c) disseminating the fungus spore to the grains (Jouany, 2007). As the majority of the cereals, the maize landraces can be affected by fungus and its secondary metabolite, the mycotoxins. *Fusarium* sp is one of the main fungi that affect the crop and is responsible for the production of the zearalenone mycotoxin (Edwards, 2004). The contamination by fungus and its mycotoxins can cause a sort of problems related with health and economic, such as the losses of agricultural products, low yield and death of animals, illnesses in humans and the rejection of the product by the consuming market (Diniz, 2002).

Different researchers have studied the physical characteristics of the grains and the proportion of the hard and soft endosperm, as well as its contamination by fungi and mycotoxins, but few works deal with the relation between the physical and mechanical characteristics with the contamination of the grains by fungi. A more accurate study in relation to the quality of the seeds produced by the small farmers of the Southern region of Brazil will be useful as strategy to evaluate the best methods to conserve the grains, as well to adding value to the production and commercialization of the local maize varieties. Due to the concern of safety food supply and aiming to contribute with the quality improvement of the maize landraces produced in the Southern region of Brazil, this work had as a main objective the study of the relation between the physical and mechanical characteristics of the grain and its contamination by *Fusarium* sp and zearalenone.

MATERIALS AND METHODS

The twenty varieties of maize landraces cultivated by farmers from Paraná and Rio Grande do Sul states in the Southern of Brazil was sowed at the State University of Ponta Grossa school farm in the 2006/2007 crop season. The farm is located at 25°16' South latitude and 50°16' West longitude with 900 m of altitude. After the manual harvest and mechanical threshes, the samples used for the rupture force tests and the fungus identification were stored at 22 ± 2 °C for 7 days under

environmental conditions with relative humidity of the air of approximately 70 %. For the further analysis, the samples were drought to a moisture level of $10 \pm 1\%$, and stored in closed polyethylene tereftalate (PET) containers under environmental temperature and humidity.

Real density

To establish the real density (d) of the analyzed samples, a pycnometer method recommended by Correa et al. (2002) was used. As a liquid supplement of the volume the xylene was used, in duplicate.

Force of rupture

A sub-sample of 10 grains of each variety was randomly chosen and used. The assays of rupture force (FR) was made following the methodology proposed by Sandhu; Singh and Malhi (2007) in substitution of the equipment used for the authors by a Shimadzu mark model AG I, with load cell with the maximum capacity of 10 kN. The values were expressed in kN.

Index of flotation

The flotation index (FI), that estimates the relative density of the grains, and in indirect way measure the hardness of endosperm, obtained in agreement with the methodology proposed by Lozano-Alejo et al. (2007). The scale used for the classification of the maize hardness was cited for Salinas, Martinez and Gómez (1992), where in values of FI of 0-12% the maize is considered very hard, hard from 13 to 37%, intermediary from 38 to 62%, soft from 63 to 87% and above of 87% is very soft.

Fungi identification

The used methodology was the "Blotter test" proposed by the ISTA (1981), which samples of grains (400 grains for variety) were incubated for seven days under a temperature of 22 ± 2 °C, submitted to a photo-period of 12h light/12h dark. Later, the grains of each variety were individually analyzed under a stereoscopic microscope. Samples of *Fusarium* sp were cultured on PDA plates and incubated in chambers at 22 ± 2 °C.

Determination of zearalenone

The presence of the zearalenone mycotoxin was evaluated by thin layer chromatography using the methodology proposed by Soares and Rodriguez-Amaya (1989).

Statistical Analysis

For the analysis of correlation and analysis of variance the Assisat 7.5 BETA program was used. For the interpretation of the rupture force data the programs Trapezium and Origin 6.1 were used. For the analysis of the main components the program Pirouette 4.0 was used.

RESULTS AND DISCUSSION

The analyzed varieties of maize presented significant difference ($p < 0.01$) between its physical and mechanical characteristics.

The rupture force level of the kernels were between 267.8 N and 507.4 N. These values were similar to the ones found for Verna and Prassad, (2000) and Sandhu, Singh and Malhi (2007) in hybrid maize.

According to Dorsey-Redding, Hurburgh, Johnson

and Fox (1991) who have analyzed hybrid varieties of maize in the United States, the difference of the force of rupture between varieties and even between grains of the same variety, occurs due to the differences in the hardness of the grain, in the proportion between hard and soft endosperm and its relation to the environmental crop conditions. The flotation index obtained, that predicts the proportion between hard and soft endosperm, was between 22% and 60% (Table 1). This result is below of those described by Peplinski et al. (1989) in hybrid maize in the United States and above of those presented for Duarte et al. (2007), for hybrid maize in the condition of second crop season in Brazil.

The quality of the seed can be affected by the mechanics injury, being that supreme with a higher value of the necessary force until the rupture, they will have little possibilities if present a fungus contamination (Andrade et al., 1999).

Table 1 - *Fusarium* sp contamination and zearalenone and flotation index for landraces varieties analyzed Samples followed for by the same letter in the same column do not differ statistical between itself to the level of 5% for the test of Scott Knott. ND. Not detected.

Varieties	<i>Fusarium</i> sp (%)	FI (%)	Zea ($\mu\text{g kg}^{-1}$)
1	24.7	38 b	64 i
2	15.2	43 a	ND
3	16.7	38 b	ND
4	11.7	37 b	640 a
5	21.5	53 a	256 e
6	11.5	32 c	ND
7	22.2	39 b	363 c
8	22.2	40 b	170 g
9	7.5	29 c	85 h
10	10.7	19 d	ND
11	6.7	32 c	ND
12	10.2	32 c	640 a
13	17.7	30 c	320 d
14	8.2	32 c	192 f
15	12.0	47 a	448 b
16	5.5	25 d	64 i
17	10.5	29 c	192 f
18	18.7	40 b	256 e
19	11.7	43 a	ND
20	24.0	44 a	ND
CV (%)	-	9.1	1.4

According to the classification presented by Salinas, Martinez and Gómez (1992), 60% of the analyzed maize can be fit in the group that possess a hard texture endosperm and 40% with endosperm of intermediate texture, being statistically different to the 1% level. The higher flotation index of the grain with soft endosperm is explained by the fact of it has a higher porosity in its endosperm (Martinez-Herrera and Lachance, 1979).

The density of the grains varied between 1.22 g mL^{-1} to 1.31 g mL^{-1} . These results were similar to those cited for Correa et al. (2002), when analyzed grains of hybrid maize from Brazil and the United States. According to them, the density is a preponderant factor to predict the proportion of hard and soft endosperm in the grain, that is, the hardness of the grain.

The correlation between the variable rupture force/flotation index, rupture force/density and

density/flotation index were significant, presenting the following values $r=-0.52$ ($p<0.01$), $r=0.46$ ($p<0.05$) and $r=-0.48$ ($p<0.05$) respectively. This is an indication that the higher is the proportion of hard endosperm, higher will be the necessary force until the rupture of the grain. Similar data were given by Jamin and Flowers (1998), Pomeranz et al. (1984) and Kirleis and Stroshine (1990), in hybrid maize from the United States.

The level of contamination by *Fusarium* sp was between 5.5% and 24.75% (Table 1), varying significantly between the analyzed varieties.

Through the correlation analysis was possible to verify that the percentage of *Fusarium* sp was higher in the varieties with a higher index of flotation ($r= 0.59$). This probably happens due to the fact of these grains have a higher proportion of hard endosperm.

The grain of hybrid maize with a higher proportion of soft endosperm has smaller necessary force until the rupture when compared to one that has a higher bigger ratio of hard endosperm (Jamin and Flowers, 1998). It also has a higher probability of attack by insects, due to easiness of penetration in endosperm (Bútron et al., 2009).

According to Buerstmayr et al. (2003), the genetic resistance of the grain to the infection by *Fusarium* sp depends on two factors: resistance to the penetration of hyphae in the grain and resistance to the fungic development in endosperm of the grain. In a study carried out by Ono et al. (2000) moldy grains have a positively correlation with the number of units of colony of *Fusarium* sp, indicating that grains with damages in pericarp

is a way of fungi contamination.

It was verified the presence of zearalenone in 75% of the samples, in concentrations that varied from 50 to 640 $\mu\text{g kg}^{-1}$. Brazil does not have specific legislation for the contamination by zearalenone in food, but the most of the countries, which has legislation for this mycotoxin, considers the limits between 100 to 200 $\mu\text{g kg}^{-1}$ to cereals and its by-products. From the analyzed samples, 50% presented contamination for zearalenone above of 100 $\mu\text{g kg}^{-1}$.

The results found are above those cited in literature by Machinski Jr et al. (2001) and Mie Kawashima and Valente Soares (2006), for hybrid maize of Brazil, but are similar to those ones cited by Briones-Reyes, Gómez-Martínez and Cueva-Rolón (2007), for hybrid maize with origin in Mexico.

According to Birzele, Prange and Kramer (2000) and Hashimoto et al. (2003), the production of mycotoxins for *Fusarium* sp, will be higher in conditions of humidity above of 20%, below of this level the fungus development occurs, but the mycotoxin production is suppressed. The presence of fungus does not imply in the presence of the mycotoxins, which is only produced in specific conditions which the grain is submitted.

Through the analysis of main components, it was verified that about 50% of the samples had been grouped around characteristics of FI and contamination for *Fusarium* sp, as verified in the samples detached in the Figure 1a, indicating that the similarity of these samples is mainly related by the correlation between these two characteristics.

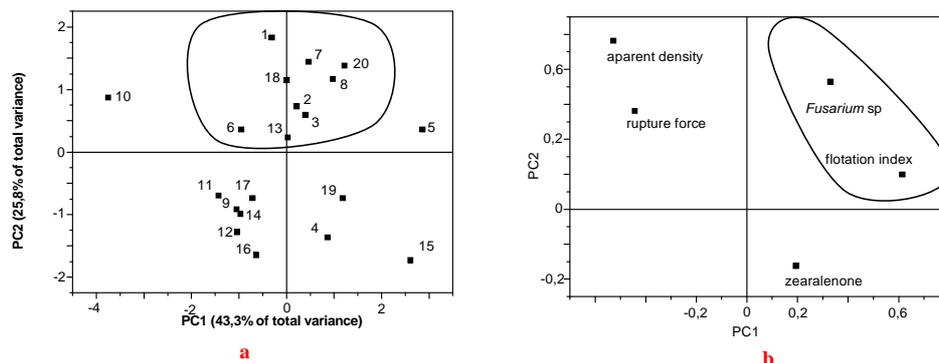


Figure 1 - Graph of scores (a) and loadings (b) for landraces maize samples analyzed for PCA, the correlation between the physical characteristics and contamination by *Fusarium* sp and zearalenone.

The remains samples are not explained by the physical characteristics. These data indicate that

the landraces varieties of maize with a higher proportion of soft endosperm can present a higher

level of contamination by *Fusarium* sp, probably caused for the biggest easiness of attack by insects in grains with this type of endosperm, since these grains are the most susceptible to pressure and break. To minimize the grain contamination problem by fungi lived by small farmers, it is suggested the use of landraces varieties with the texture of endosperm predominantly hard.

CONCLUSIONS

The all studied landraces varieties possess endosperm of the hard and intermediate type, therefore less susceptible to fungic contaminations. The relation between the physical and mechanical characteristics of the grains of the landraces varieties studied and the contamination by *Fusarium* sp, suggests the higher is the soft ratio of the grains endosperm, the more susceptible to the contamination it will be. Therefore, more easily it can be attacked by insects, facilitating the contamination. The contamination for zearalenone was not related to the contamination by *Fusarium* sp and the hardness of endosperm. Finally it concludes that the physical and mechanical characteristics presented by the landraces varieties of the present study are similar to those related in literature for hybrid maize.

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RESUMO

Este trabalho teve por objetivos verificar as características físicas, mecânicas, contaminação por *Fusarium* sp e por zearalenona e suas relações, em vinte variedades crioulas de milho da região sul do Brasil. Verificou-se que das amostras analisadas, 60% foram consideradas como possuidoras de endosperma do tipo duro e 40% com endosperma do tipo intermediário. As variáveis físicas e mecânicas se correlacionaram, indicando que quanto maior a proporção de endosperma vítreo, mais denso e maior a força

necessária até a ruptura do grão. A contaminação por *Fusarium* sp esteve entre 5,5% e 24,75% nos grãos analisados, correlacionando-se positivamente com o índice de flotação, indicando que as variedades crioulas de milho com uma maior proporção de endosperma macio podem apresentar uma maior contaminação por *Fusarium* sp, sugerindo-se a utilização de variedades crioulas com a textura do endosperma predominantemente vítrea. A presença de zearalenona foi verificada em 75% das amostras, em concentrações que variaram de 50 a 640 µg kg⁻¹.

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